

Toxicology, Structure–Function Relationship, and Human and Environmental Health Impacts of Polychlorinated Biphenyls: Progress and Problems

by Stephen Safe

Polychlorinated biphenyls (PCBs) are industrial compounds that have been detected as contaminants in almost every component of the global ecosystem including the air, water, sediments, fish, and wildlife and human adipose tissue, milk, and serum. PCBs in commercial products and environmental extracts are complex mixtures of isomers and congeners that can now be analyzed on a congener-specific basis using high-resolution gas chromatographic analysis. PCBs are metabolized primarily via mixed-function oxidases into a broad spectrum of metabolites. The results indicate that metabolic activation is not required for PCB toxicity, and the parent hydrocarbons are responsible for most of the biochemical and toxic responses elicited by these compounds. Some of these responses include developmental and reproductive toxicity, dermal toxicity, endocrine effects, hepatotoxicity, carcinogenesis, and the induction of diverse phase I and phase II drug-metabolizing enzymes. Many of the effects observed for the commercial PCBs are similar to those reported for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Structure–function relationships for PCB congeners have identified two major structural classes of PCBs that elicit “TCDD-like” responses, namely, the coplanar PCBs (e.g., 3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB and 3,3',4,4',5,5'-hexaCB) and their mono-*ortho* coplanar derivatives. These compounds competitively bind to the TCDD or aryl hydrocarbon (Ah) receptor and exhibit Ah receptor agonist activity. In addition, other structural classes of PCBs elicit biochemical and toxic responses that are not mediated through the Ah receptor. The short-term effects of PCBs on occupationally exposed humans appear to be reversible, and no consistent changes in overall mortality and cancer mortality have been reported. Recent studies have demonstrated that some developmental deficits in infants and children correlated with *in utero* exposure to PCBs; however, the etiologic agent(s) or structural class of PCBs responsible for these effects have not been delineated. In contrast, based on a toxic equivalency factor approach, the reproductive and developmental problems in certain wildlife populations appear to be related to the TCDD-like PCB congeners.

Introduction

Polychlorinated biphenyls (PCBs) are industrial compounds that are readily synthesized via the ferric ion or iron-catalyzed chlorination of biphenyl (*1–3*). Commercial PCBs were manufactured by several companies and marketed worldwide under a variety of trade names, which included Aroclor (Monsanto, U.S.A. and United Kingdom), Clophen (Bayer, Germany), Phenoclor and Pyralene (Prodelec, France), Kanechlor (Kanegafuchi, Japan), Santotherm (Mitsubishi-Monsanto, Japan), and Fenclor (Caifaro, Italy). In addition, these products were also manufactured in the U.S.S.R. and Czechoslovakia. Commercial PCBs are synthesized, graded, and marketed according to their percentage by weight chlorine content. For example, the commercial Aroclors named 1221,1232,1242,1248,1254,1260,1262, and 1268 contain 21,32,42,48,54,60,62, and 68 % by weight chlorine, respectively, as indicated by the last two num-

bers of their four-digit name. Aroclor 1016 is another PCB product that resembles Aroclor 1242 in composition except that the penta- and hexachlorobiphenyl components have been removed.

The chemical properties of the commercial PCB mixtures are dependent on their degree of chlorination; for example, the lower chlorinated mixtures are mobile, colorless oils, whereas the higher chlorinated Aroclors 1262 and 1268 are an immobile, viscous liquid and an amorphous solid, respectively. The estimated worldwide production of PCBs is approximately 1.5 million metric tons (2). The widespread industrial utilization of PCB products was due to their variable physical properties (i.e., liquid to solid), chemical stability, dielectric properties, inflammability, and miscibility with organic solvents. PCBs have been used as heat transfer fluids, hydraulic lubricants, dielectric fluids for transformers and capacitors, plasticizers, wax extenders, adhesives, organic diluents, dedusting agents, pesticide extenders, cutting oils, carbonless reproducing paper and flame retardants. The widespread use of PCBs coupled with improper disposal practices has resulted in their introduction into the environment. Moreover, due to the relative stability of the more highly chlorinated PCBs and their lipophilicity, PCBs are widely

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distributed and transported throughout the environment, and their residues have been identified in air, water, aquatic and marine sediments, fish, and wildlife and human adipose tissue, serum, and milk (2). PCBs, along with DDT and some of its metabolites, are among the most widely identified organic contaminants found in extracts from almost all environmental samples.

PCB Analysis and Environmental Impact

The chlorination of biphenyl is not highly regiospecific and results in the formation of a complex mixture of isomers and congeners (1,3). The composition of the different Aroclor mixtures is dependent on their degree of chlorination because there is generally an increase in the more highly chlorinated congeners with increasing chlorination. Early analytical studies on commercial PCBs using gas liquid chromatography (GLC) clearly demonstrated both the complexity of these mixtures and the differences in chromatographic patterns, which were dependent on their degree of chlorination (1). The analysis of environmental extracts by low-resolution packed column GLC utilizes specific peaks present in the chromatograms of the commercial PCBs (or reconstituted mixtures of different products) to estimate the PCB levels (4). This method relies heavily on the limited pattern recognition of selected peaks; however, in many cases the GLC pattern of PCBs in many environmental extracts do not resemble the corresponding patterns observed for any of the commercial PCBs (5-9).

The problems associated with PCB analysis have been resolved with the development of high-resolution capillary columns and the synthesis of all 209 PCB isomers and congeners as reference standards (10). Safe and co-workers (6,11) first described the high-resolution GLC analysis of a commercial PCB, Aroclor 1260, and an extract from a composite human milk sample. At least 88 different congeners were identified in Aroclor 1260, whereas only 55 individual PCBs were identified in the human milk sample. Surprisingly, 11% of the total PCBs identified in the latter sample were the lower chlorinated 2,4,4'-trichlorobiphenyl (triCB, 8.8%) and 2,4,4',5-tetrachlorobiphenyl (tetraCB, 2.2%), congeners that are minor to trace components of Aroclor 1260. The reason for the persistence of the lower chlorinated PCBs, particularly 2,4,4'-triCB, in human tissues is unknown. Several PCB congeners identified in the milk sample, including 2,2',4,4',5,5'-hexachlorobiphenyl, (hexaCB), 2,2',3,4,4',5'-hexaCB, 2,2',3,3',4,4',5-heptachlorobiphenyl (heptaCB), and 2,2',3,4,4',5'-heptaCB, are major components of Aroclor 1260. 2,3,3',4,4',5-HexaCB is also a major PCB component of the human milk extract but is a minor component of Aroclor 1260. It was also of interest to note that several PCB congeners that are major components (22.8% of total) of Aroclor 1260 [2,2',3,5',6-pentaCB (2.7%); 2,2',3,4',5',6-hexaCB (7.4%); 2,2',3,4,5,5',6-heptaCB (4.1%); 2,2',3,3',4,5,6'-heptaCB (5.5%); 2,2',3,3',4,4',5,6-octachlorobiphenyl (3.1%)] are present in only trace levels (0.81% of total) in the human milk extracts. With the exception of 2,2',3,3',4,4',5,6-octachlorobiphenyl, all, of these compounds possess a 2,3,6-trichloro- or 2,5-dichloro-substitution pattern on at least one of their phenyl rings and,

because of the two adjacent unsubstituted carbon atoms, rapid metabolic degradation of the congeners would be expected (11). The failure of the octachlorobiphenyl to accumulate in humans may be due to uptake factors, as the persistent higher chlorinated PCBs (Cl₈-Cl₁₀) are not major contaminants in human tissues.

Schulz et al. (12) have recently reported the complete characterization of the PCB congeners in Aroclors 1016, 1242, 1254, and 1260, Clophens A30, A40, A50, and A60 by multidimensional GLC-electron capture detection. A total of 132 of the 209 possible congeners were identified in these mixtures, and these data will be useful for carrying out the unambiguous quantitative analysis of PCBs in extracts from different environmental matrices. Several studies have already reported the high-resolution GLC analysis of PCBs in the environment, and some of the results were summarized by McFarland and Clarke (13). The uptake and retention of individual PCB congeners is dependent on the animal species and the congener composition of the various local sources of PCB contamination. Inspection of the analytical data (13) indicates that there are major differences in the PCB composition in fish, wildlife, and humans; however, the congeners that appear most frequently in all analytes are 2,2',3,4,4',5'-hexaCB, 2,2',4,4',5,5'-hexaCB and 2,2',3,4,4',5,5'-heptaCB. The potential adverse environmental and human health impacts of PCBs is dependent on several factors including the overall levels of PCB exposure, the toxicities of the individual congeners present in the mixture, and their interactive effects. Some of these factors and their importance will be addressed in this review.

Role of Metabolism in the Toxicity of PCBs

The toxic effects elicited by many different structural classes of chemicals require their metabolism into activated intermediates, which subsequently alkylate critical cellular macromolecular targets (14,15). The metabolic activation process for many different halogenated hydrocarbons often involves cytochrome P-450-dependent oxidation or transformation of the hydrocarbon to form arene oxide or radical intermediates that are capable of forming covalent adducts with cellular constituents. Early studies with PCBs clearly demonstrated that these hydrocarbons are converted into polar hydroxylated metabolites and the results of studies with individual PCB congeners are consistent with the metabolic scheme illustrated in Figure 1 (11,16-19). PCBs are substrates for P-450 enzyme-catalyzed oxidation into highly reactive arene oxide intermediates, which in turn rearrange to give phenolic metabolites or are further metabolized via various metabolic pathways. For example, epoxide hydrolase and glutathione S-transferases catalyze the conversion of arene oxides into dihydrodiols and glutathione conjugates, respectively. In addition, the phenolic metabolites can undergo further oxidative metabolism and/or conjugation, and glutathione conjugates are precursors of a number of sulfur-containing metabolites including the methylsulfonyl compounds.

The rate of PCB metabolism and the regioselectivity of the initial arene oxide formation are dependent on several factors, which include a) the degree of ring chlorination, b) the chlorine ring substitution pattern, and c) the pattern and levels of P-450 isozymes and other drug-metabolizing enzymes in the target organ. For example, phenobarbital-induced P-4502 isozymes

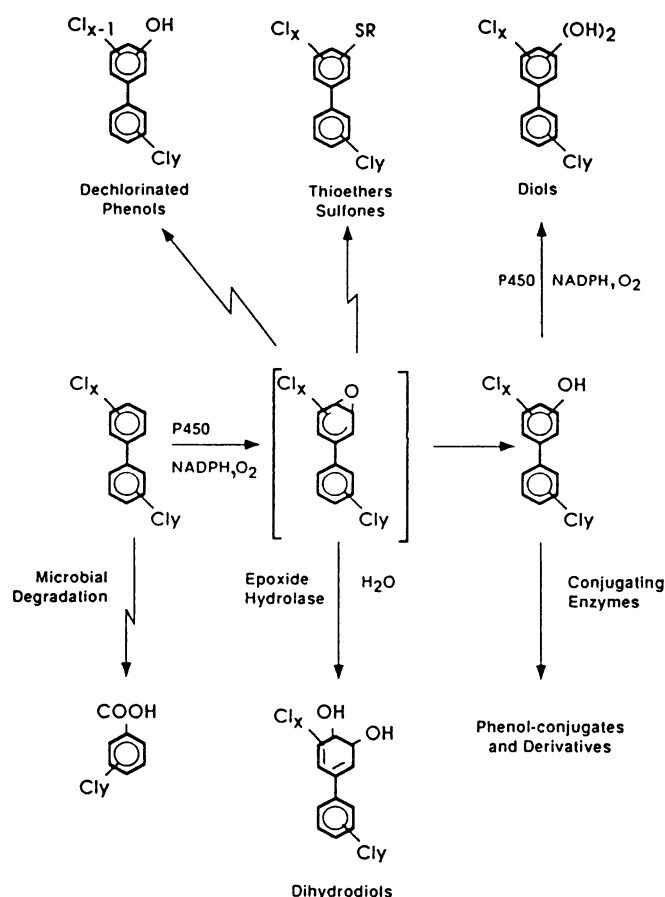


FIGURE 1. Proposed metabolic scheme for the PCBs.

catalyze the metabolism of dichlorobiphenyls with *diortho* chlorine substitution, whereas P-4501 isozymes primarily metabolize dichlorobiphenyls, which do not contain any *ortho* chloro substituents. In contrast, mono-*ortho*-chloro-substituted dichlorobiphenyls are metabolized by both induced P-4501 and P-4502 isozymes (20). There is also evidence that PCBs may also be oxidized via pathways that do not involve arene oxide intermediates (21).

The biochemical and toxic responses associated with PCB metabolism have been investigated. The results of *in vivo* and *in vitro* studies show that PCBs form covalent adducts with cellular macromolecules (protein, RNA, and DNA) and induce DNA strand breaks and DNA repair (22–27). Presumably these events are dependent on the metabolism of PCBs into intermediates such as arene oxides, which alkylate cellular macromolecules. In contrast, PCBs are not bacterial mutagens (28) and in many cases those PCBs that are most readily metabolized are among the least toxic members of this class of compounds. On balance, it would appear that PCBs are not genotoxic and the metabolic activation process per se does not play a significant role in the toxicity of PCBs.

Several studies have demonstrated that the methylsulfonyl PCB metabolites (29–33) exhibit some unusual biochemical properties. For example, methylsulfonyl PCB metabolites bind to

uteroglobin, a progesterone-binding protein (34). In addition, methylsulfonyl metabolites such as 4,4'-bis(methylsulfonyl)-2,2',5,5'-tetrachlorobiphenyl accumulate in lung tissue, where it appears to bind with moderately high affinity to a lung-binding protein (35). Methylsulfonyl metabolites have been identified in human subjects and in relatively high levels in individuals accidentally exposed to PCBs in the Yusho poisoning incident (36). Because changes in lung capacity and function in humans has been associated with PCB exposure (37), it has been suggested that the methylsulfonyl PCB metabolites may play a role in the etiology of this toxic response.

Hydroxylated PCB metabolites have been identified in wildlife samples (38), and several *in vitro* studies have shown that these compounds are potent inhibitors of mitochondrial oxidative phosphorylation (39). However, a number of hydroxylated metabolites of some of the more toxic PCB congeners (e.g., 3,3',4,4',5-pentaCB) have been identified, subsequently administered to laboratory animals, and shown to be relatively nontoxic. (40–42).

Thus, although PCBs are metabolized, in part, through arene oxide intermediates to give a number of metabolic products, the evidence suggests that PCB metabolism serves primarily as a detoxification process. These results imply that the toxic and biochemical responses associated with exposure to PCB mixtures and congeners are primarily due to the parent hydrocarbons. This is in contrast to many other classes of xenobiotics including chlorinated hydrocarbons and the halogenated benzenes, in which metabolic activation plays a critical role in their toxicity (14,15).

PCB Mixtures: Biochemical and Toxic Responses

The toxic and carcinogenic responses elicited by commercial PCB mixtures have been extensively investigated in laboratory animals, mammalian cells in culture, and in exposed human populations (3,43–47). The PCB-induced effects are dependent on a number of factors including the age, sex, and species of the test animals, the route of administration, the duration of exposure, and the chlorine content of the PCB mixture. For example, in Sprague-Dawley rats fed a diet of Aroclor 1260 for 2 years (100 ppm for 16 months and 50 ppm for 8 months), the incidence of hepatocellular adenocarcinomas was 51% in female rats and 0% in male rats (48). Schaeffer and co-workers (49) used male Wistar rats as model for determining the effects of chronic feeding of 100 ppm of Clophen A42 and Clophen A60. After 800 days, the incidence of hepatocellular carcinomas in the Clophen A60, Clophen A30 and control rats was 61, 3, and 2%, respectively. This study illustrated the significant differences between the hepatocarcinogenicity of the more potent, higher chlorinated Clophen A60 PCB (60% Cl by weight) versus the lower chlorinated Clophen A30 (42% Cl by weight). Moreover, because the chemical composition of Aroclor 1260 and Clophen A60 are similar, the results of the chronic feeding studies by Norback and Weltman (48) and Schaeffer and co-workers (49) also indicate intraspecies differences in male rat susceptibility to the hepatocarcinogenicity of PCBs.

The acute, subacute, and subchronic toxic responses to commercial PCBs are characterized by LD_{50} values that are usually $> 1000 \text{ mg/kg}$, an extensive array of hepatotoxic effects, body

weight loss in some studies, some immunotoxicity and effects on the gastrointestinal system, dermal lesions (primarily in primates), and developmental and reproductive toxicity. In chronic feeding studies, most of the above toxic responses were observed; in addition, several studies have reported that PCBs administered alone are carcinogenic, with the liver being the primary target site (47–49). It has also been reported that mink and monkeys were among the most sensitive species to the toxic effects of commercial PCBs (50–53).

The biochemical properties of PCB mixtures have also been extensively investigated, and one of the hallmarks of PCB exposure is the induction of phase I and phase II drug-metabolizing enzymes (43,46). For example, several commercial PCB mixtures induce the phase II enzyme systems, epoxide hydrolase, glucuronosyl transferase, glutathione *S*-transferases and reductase (54–56). In addition, PCB mixtures induce a broad spectrum of P450-dependent monooxygenase enzyme activities including several polynuclear aromatic hydrocarbon hydroxylases, steroid hydroxylases, *O*-dealkylases, several haloaromatic hydroxylases, *N*-dealkylases, barbiturate hydroxylases, and the oxidative metabolism of a host of other substrates including aflatoxin, warfarin, aldrin, acetanilide, etc. (43,46,57,58). Early studies on the effects of drug-metabolizing enzyme inducers showed that phenobarbital (PB) and 3-methylcholanthrene (MC) were prototypes of two different classes of mixed-function oxidase inducers (59–61). In contrast, the induction activities of commercial PCBs such as Aroclor 1254 resembled those of PB plus MC (coadministered), and Aroclor 1254 and other PCB mixtures were designated as mixed-type inducers (62–64). Subsequent research has shown that the differences in PB and MC as inducers were due to their preferential induction of different P-450 isozymes (65–68). Phenobarbital induces CYP2B1 (P-450b) and CYP2B2 (P-450e), MC induces CYP1A1 (P-450c) and CYP1A2 (P-450d), and both chemicals induce low levels of CYP2A1 (P-450a). Not surprisingly, Aroclor 1254 induced all five P-450 isozymes. It was therefore of interest to determine which PCB congeners were responsible for not only for the induction activities of the commercial PCB mixtures but also for the toxic effects elicited by the compounds.

PCB Congeners: Structure–Function Relationships

The structure–activity relationships (SARs) for several different structural classes of PCBs have been carried out, and the results of these studies have delineated many of the PCB congeners that are responsible for the toxic and biochemical activities associated with the commercial and environmental mixtures (69–73).

PCBs That Exhibit TCDD-like Activity

Coplanar Congeners. Structure–induction and structure–toxicity relationships for PCBs have demonstrated that three PCB congeners (Fig. 2), namely, 3,3',4,4'-tetrachlorobiphenyl (tetraCB), 3,3',4,4',5-pentachlorobiphenyl (pentaCB) and 3,3',4,4',5,5'-hexachlorobiphenyl (hexaCB) elicit the same spectrum of toxic and biochemical responses observed for TCDD and related halogenated aromatic hydrocarbons (43,46,57,58,74–76).

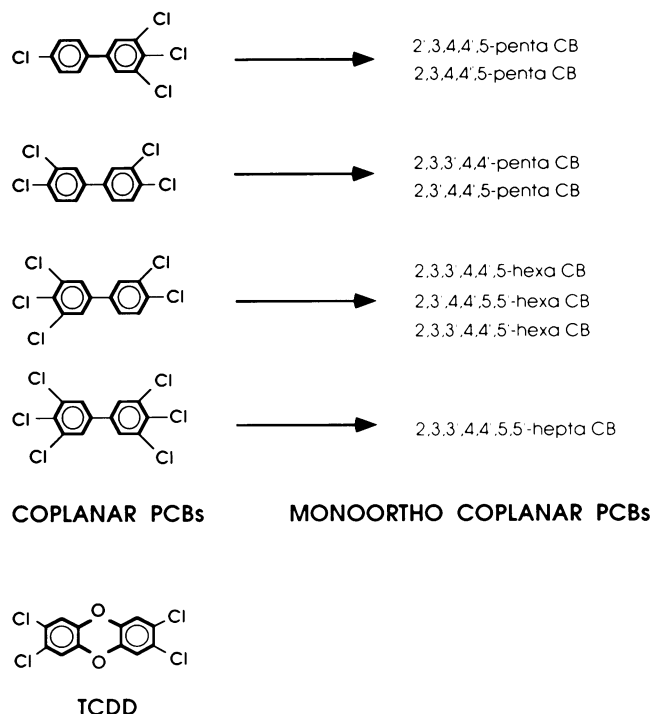


FIGURE 2. The structures of 2,3,7,8-TCDD, the coplanar PCBs, and the derived mono-*ortho* coplanar analogs.

As illustrated in Figure 2, in their coplanar conformation these three congeners and 3,4,4',5-tetraCB are approximate isostereomers of TCDD in which the Cl groups are substituted only in the lateral (*para* and *meta*) positions. Structure–activity studies have shown that two *para* (4 and 4'), two or more *meta* (3,3', and 5') and no *ortho* (2,2',6 and 6') substituents are required for maximum TCDD-like activities. The coplanar PCBs competitively displace [³H]TCDD from the cytosolic Ah receptor (77), and these compounds exhibit all the properties of Ah receptor agonists including the induction of CYP1A1 and CYP1A2 (54).

Mono-*ortho* Coplanar Congeners. Aust and co-workers (78) first demonstrated that the mono-*ortho*-substituted polybrominated biphenyl (PBB), 2,3',4,4',5,5'-hexabromobiphenyl, resembled the commercial PBB mixture, FireMaster BP-6, and Aroclor 1254 as a mixed-type inducer of rodent hepatic drug-metabolizing enzymes. Subsequent studies showed that all the mono-*ortho* coplanar PCBs (Fig. 2) were mixed-type inducers and resembled Aroclor 1254 in their pattern of drug-metabolizing enzyme induction (46,54,79–81). The mono-*ortho* coplanar PCB congeners also competitively displaced [³H]TCDD from the cytosolic Ah receptor (77), and the results of limited studies have demonstrated that this structural class of PCBs exhibits Ah receptor agonist activity (54,82–88). However, quantitative SARs have shown that the relative Ah receptor binding affinities and Ah receptor agonist activities followed the order TCDD > 3,3',4,4',5-pentaCB > 3,3',4,4'-tetraCB and 3,3',4,4',5,5'-hexaCB > mono-*ortho* coplanar PCBs.

Other PCBs As Ah Receptor Agonists. At least two additional structural classes of PCBs have been identified as Ah receptor

agonists. The 17 di-*ortho* coplanar derivatives of the 4 coplanar PCBs and 3,4,4'-trichlorobiphenyl have been synthesized and tested as inducers of rodent microsomal aryl hydrocarbon hydroxylase (AHH) activity and the associated P-450 isozymes (54,89,90). The results indicated that at relatively high doses, most of the di-*ortho* coplanar PCBs that have been tested induce AHH activity and appear to be weak Ah receptor agonists. Limited studies have also demonstrated that some of these congeners elicit other TCDD-like responses; for example, 2,2',3,3',4,4'-hexaCB and 2,2',3,4,4',5'-hexaCB are porphyrinogenic in rodents, and 2,3',4,4',5',6-hexaCB induced AHH activity and suppressed the splenic plaque-forming cell response to sheep red blood cells in C57BL/6 mice (85,91). At least one compound in this structural class, 2,2',4,4',5,5'-hexaCB, was not an Ah receptor agonist and resembled PB in its pattern of drug-metabolizing enzyme induction (54). Davis and Safe (85) also reported that at least two congeners, 2,3,3',4,5'-pentaCB and 2,3,3',4,5,5'-hexaCB, are also immunotoxic in C57BL/6 mice, and the former compound also induced hepatic microsomal AHH activity. These congeners structurally resemble mono-*ortho* coplanar PCBs in which one of the *para* substituents has been removed. These compounds and some of the di-*ortho* coplanar PCB analogs exhibit low competitive binding affinities for the Ah receptor and exhibited potencies as Ah receptor agonists that were considerably lower than the mono-*ortho* coplanar PCBs.

Biochemical and Toxic Responses of PCBs That Do Not Act through the Ah Receptor. There is interest by scientists and regulatory agencies in regulating PCBs on a congener basis (92,93). The identification and relative potencies for some of the TCDD-like congeners have been determined in several *in vivo* and *in vitro* bioassays [reviewed in Safe (46)]. However, any scheme that is devised for congener-based regulation of PCBs must also take into account the potential toxicities and adverse human and environmental health effects of other structural classes of PCB congeners. It was apparent from the initial studies with Aroclor 1254 that PCBs also resembled PB in their pattern of drug-metabolizing enzyme induction. Moreover, several individual congeners, including 2,2',4,4'-tetraCB and, 2,2',4,4',5,5'-hexaCB, have been characterized as pure PB-type inducers and structure-activity studies suggest that compounds substituted in at least two *para* and two *ortho* substituents exhibit this type of activity (95). A thorough study of the potential adverse health effects of this class of PCBs has not been determined. However, like PB, these compounds cause hepatomegaly and exhibit activity as promoters in short-term bioassays for carcinogenesis. For example, 2,2',4,4',5,5'-hexaCB promotes diethylnitrosamine-induced ATPase-deficient lesions in rat liver (95,96).

It has also been reported that other structural classes of PCBs are inducers of P-450 isozymes. For example, several highly chlorinated congeners with two or more *ortho* substituents induced CYP3A1 (P-450) in cultured rat hepatocytes (97). This P-450 isozyme is classically induced by dexamethasone and related glucocorticoids (96). The toxicological significance of this PCB-induced biochemical response is unknown. Several studies have reported the neurobehavioral toxicity of commercial PCBs and individual congeners in rodents (98-106). Although some of the observed responses have been associated with 3,3',4,4'-tetraCB, a TCDD-like congener (102-104), the

neurotoxicity of other structural classes of PCBs have been reported (105,106). For example, studies on the structure-dependent decrease in the dopamine content of PC12 cells showed that the most active congener was 2,2'-dechlorobiphenyl and other congeners with relatively high *ortho* (two to three) and *para* (up to two) but low *meta* substitution were also active (106). Many of the compounds that were active in the *in vitro* studies also caused a similar decrease in the dopamine content in brain tissue of rodents and nonhuman primates (105). These data suggest that some of these *ortho*-substituted PCBs may also play a role in the toxicities elicited by PCB mixtures. The adverse environmental and human health impacts of these more highly *ortho*-substituted PCB congeners are unknown and require further investigation.

Human Health Effects of PCBs

The human health effects of PCBs have been reviewed (45,107-110). Exposure of humans to relatively high levels of PCBs has occurred primarily in individuals working in plants that extensively used PCBs and PCB-containing equipment. Occupational exposure to PCBs can result in a broad spectrum of symptoms which include elevated serum lipid levels, increased levels of some serum enzymes, chloracne and related dermal lesions, possible hepatic damage, and respiratory problems (37,111-116). It is noteworthy that the effects of PCBs were not consistently observed in all the occupationally exposed workers, and it is possible that some of the responses may be due to the highly toxic polychlorinated dibenzofurans (PCDFs), which are present as by-products in commercial PCBs (117,118). The acute and subacute effects of PCBs associated with the Yusho and Yucheng accidents in Japan and Taiwan, respectively, will not be discussed in this review because the evidence suggests that the toxic responses observed in the victims of these exposures were due to the relatively high levels of PCDFs that were identified as by-products in the PCB-containing industrial fluid (119,120).

Epidemiological studies on several groups of workers occupationally exposed to PCBs have been reported (121-124). The results from these studies were variable and did not show any consistent trends with respect to overall mortality or deaths from specific cancers. For example, in a study by Bertazzi and co-workers (123) on a group of capacitor workers, cancer deaths were elevated in the male and female workers and overall mortality was also increased in the latter group. Their results suggested that increased cancer in lymphatic and hemopoietic tissues and the gastrointestinal tract were associated with PCB exposure. In contrast, a second study (122) showed that there was a decreased overall mortality and mortality from cancer in 2588 capacitor workers. There were some increases in specific cancers in this group (e.g., liver, gall bladder, and biliary tract); however, due to confounding factors it was difficult to associate these specific cancers with PCB exposure. Thus, at the present time, the results suggest that the adult human population most highly exposed to PCBs does not show any consistent increase in mortality or specific cancers. However, because most of the epidemiology reports were limited due to the size of the study group or the number of deaths, the long-term effect of occupational exposure to PCBs will require further monitoring of the worker population. Interestingly, Taylor and co-workers (125,126) have reported that small but significant decreases in

birth weight were observed in infants born to women employed in capacitor-manufacturing plants. This suggests that infants may be at risk from *in utero* exposure to PCBs.

The effects of PCBs on infant growth and development have been investigated in two populations, namely, a group of children in Michigan whose mothers consumed moderate quantities of fish and a group of North Carolina children (127,128). The levels of PCBs in both study groups were relatively low and resembled those observed in most normal populations. In one study (128), birth weight, head circumference, and neonatal jaundice showed no relationship to the levels of PCBs or DDE in human milk. In contrast, lower birth weights and a small head circumference correlated with PCB exposure as measured by fish consumption or cord serum PCB levels (127). The reason for the differences in the results of these studies have not been determined. Despite these differences, there were a number of similarities between the results obtained from the Michigan and North Carolina groups. Prenatal exposure to PCBs was correlated with poorer performance on the Brazelton Neonatal Behavior Assessment Scale and on the psychomotor index of the Bayley Scales of Infant Development by infants from both study groups (127–130). Moreover, in the Michigan fish-eating group, exposure to PCBs (high cord serum levels or maternal fish consumption) also correlated with poorer performance on Fagan's Visual Recognition Memory Test (131). In a subsequent follow-up study in the children at 4 years of age, the poorer short-term verbal and quantitative memory function in both was related to the PCB concentrations in cord serum (132). Moreover, their growth rate and reduced activity based on composite ratings also correlated with PCB levels (133).

Comparable effects on infant and child development have also been observed in the offspring of women exposed to PCBs, PCDFs, and related compounds in the Yucheng poisoning incident (134). There are several unresolved problems with respect to the observed developmental deficits and the etiology of these effects. For example, are the same developmental problems associated with the Michigan, North Carolina, and Yucheng groups; what is the duration of these effects; are these problems in the Michigan and North Carolina children associated with PCBs or an as yet unidentified group of lipophilic contaminants; are the PCBs, PCDFs, or both groups of compounds etiologic agents in the offspring of the Yucheng mothers; if the PCBs are etiologic agents, are the developmental problems associated with dioxinlike Ah receptor agonists or other structural classes of PCBs? These questions must be addressed in order to resolve the possible (but not proven) role of PCBs on the health of a potentially sensitive group: infants and children.

Risk Assessment of PCBs on a Congener-Specific Basis: Development of Toxic Equivalency Factors

The development of toxic equivalency factors (TEFs) for the risk assessment of pentachlorodibenzo-*p*-dioxin (PCDD) and PCDF mixtures has been adopted worldwide by several regulatory agencies (46,135–139). This approach takes advantage of the common Ah receptor-mediated mechanism of action for the PCDDs and PCDFs and uses the expected structure–activity relationships for these congeners to determine their potencies

Table 1. Relative toxic potencies and proposed TEFs for PCB congeners (46).

Congener	Potency range ^a (<i>in vivo</i> and <i>in vitro</i>)	TEF ^b
3,3',4,4',5-PentaCB	0.3–0.0006	0.1
3,3',4,4',5,5'-Hexa CB	0.1–0.0012	0.05
3,3',4,4'-TetraCB	0.009–0.00008	0.01
Mono- <i>ortho</i> coplanar PCBs	0.00045–0.0000014	0.001
Di- <i>ortho</i> coplanar PCBs	0.00002	0.00002

Abbreviations: TEF, toxic equivalency factor; PCB, polychlorinated biphenyl.

^aThe potencies of the individual congeners were determined relative to 2,3,7,8-TCDD for several different Ah receptor-mediated responses.

^bProposed by Safe (46)

relative to a standard toxin, 2,3,7,8-TCDD. The PCDD/PCDF extracts from most environmental samples primarily contain the 2,3,7,8-substituted tetra-octachlorinated compounds, and individual TEF values have been derived for all of these congeners. The validation of the TEF approach for risk assessment of PCDDs and PCDFs has been reviewed (46), and there is strong evidence that this approach is useful for predicting TCDD-like responses.

Tanabe and co-workers (140–144) first used a TEF approach for assessing the TCDD or toxic equivalence (TEQ) of PCBs in the commercial mixtures and in environmental extracts (note: $TEQ = \sum [PCDD \times TEF] + \sum [PCDF \times TEF]$). The TEF values adopted for the coplanar and mono-*ortho* coplanar PCBs were derived from the relative potencies of these compounds as inducers of AHH or ethoxyresorufin *O*-deethylase (EROD) activities in rat hepatoma H-4-II E cells compared to 2,3,7,8-TCDD as the toxic reference standard (145).

Recently, Safe (46) summarized the TEF values for the coplanar and mono-*ortho* coplanar PCBs (Table 1) and, based on the range of values, the following TEFs were proposed: 3,3',4,4',5-pentaCB, 0.1; 3,3',4,4',5,5'-hexaCB, 0.05; 3,3',4,4'-tetraCB, 0.01; mono-*ortho* coplanar PCBs, 0.001. These values are highly conservative and will require modification as new data become available. In addition, it should be stressed that the TEF approach applies only to those congeners that act through the Ah receptor and is applicable only to TCDD-like responses.

One of the earliest applications of the TEF approach was used to determine the contribution of PCB congeners to the total TEQs in samples containing PCBs, PCDDs, and PCDFs (140–144, 146–153). Initial results indicate that in most environmental extracts, PCBs contributes a significantly higher percentage of the total TEQs than the PCDD plus PCDFs (combined). A recent paper by Dewailly and co-workers (146) identified six major coplanar and mono-*ortho* coplanar PCBs in human milk (3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB, 3,3',4,4',5,5'-hexaCB, 2,3,3',4,4'-pentaCB, 2,3,3',4,4',5-pentaCB, and 2,3,3',4,4',5-hexaCB), and the TEQ value for these congeners derived from the TEF values in Table 1 was 37.76 ppt. In contrast, the TEQ value for all the 2,3,7,8-substituted PCDDs and PCDFs was 13.22 ppt. Thus, the PCBs constituted 74% of the total TEQs in the human milk samples from the province of Quebec. Recent studies on extracts from Great Lakes fish and birds (147–153) have reported that 3,3',4,4',5-pentaCB and 2,3,3',4,4'-pentaCB were the major toxic PCB congeners present, and the PCBs contributed significantly more to the TEQs than the PCDDs plus PCDFs. Moreover, there was a correlation between the reproductive failure in the wildlife populations and the PCB-TEQs.

These results clearly demonstrate the utility of the TEF approach for risk assessment of PCBs in some environmental samples and show their possible etiologic role in some adverse effects on wildlife population. Future studies should focus on further evaluation and validation of the TEF approach for the risk assessment of PCBs which exhibit TCDD-like activities. In addition, the possible role of other structural classes of PCBs on PCB-induced toxicosis must be evaluated.

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REFERENCES

- Hutzinger, O., Safe, S. and Zitko, V. The Chemistry of PCBs. CRC Press, Boca Raton, FL, 1974.
- De Voogt, P., and Brinkman, U.A.T. Production, properties and usage of polychlorinated biphenyls. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd ed. (R. D. Kimbrough and A. A. Jensen, Eds.), Elsevier-North Holland, Amsterdam, 1989, pp. 3-45.
- Safe, S., and Hutzinger, O. Polychlorinated Biphenyls (PCBs). Mammalian and Environmental Toxicology. Environmental Toxin Series, Vol. 1. Springer-Verlag, Heidelberg, 1987.
- Webb, R. G., and McCall, A. C. Quantitative PCB standards for electron capture gas chromatography. Chromatogr. Sci. 11:366-373 (1973).
- Mullin, M., Sawka, G., Safe, L., McCrindle, S., and Safe, S. Synthesis of octa- and nonachlorobiphenyl isomers and congeners and their quantitation in commercial polychlorinated biphenyls and identification in human breast milk. J. Anal. Toxicol. 5: 138-142 (1981).
- Safe, S., Safe, L., and Mullin, M. Polychlorinated biphenyls (PCBs)—congener-specific analysis of a commercial mixture and a human milk extract. J. Agric. Food Chem. 33:24-29 (1985).
- Wolff, M. S., Thornton, J., Fischbein, A., Lillis, R., and Selikoff, I. J. Disposition of polychlorinated biphenyl congeners in occupationally exposed persons. Toxicol. Appl. Pharmacol. 62: 294-308 (1982).
- Wolff, M. S., Fischbein, A., Rosenman, K. D., and Levin, S. M. Pattern designation of PCBs in human samples. Chemosphere 15:307 (1986).
- Borlakoglu, J. T., Wilkins, J. P. G., Walker, C. H., and Dils, R. R. Polychlorinated biphenyls (PCBs) in fish-eating sea birds—I. Molecular features of PCB isomers and congeners in adipose tissue of male and female razorbills (*Alca torda*) of British and Irish coastal waters. Comp. Biochem. Physiol. 97:151-160 (1990).
- Mullin, M. D., Pochini, C. M., McCrindle, S., Romkes, M., Safe, S., and Safe, L. High-resolution PCB analysis: the synthesis and chromatographic properties of all 209 PCB congeners. Environ. Sci. Technol. 18: 468-476 (1984).
- Safe, S. Polyhalogenated aromatics: uptake, disposition and metabolism. In: Halogenated Biphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd ed. (R. D. Kimbrough and A. A. Jensen, Eds.), Elsevier-North Holland, Amsterdam, 1989, pp. 51-69.
- Schulz, D. E., Petrick, G., and Duinker, J. C. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. Environ. Sci. Technol. 23: 852-859 (1989).
- McFarland, V. A., and Clarke, J. U. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ. Health Perspect. 81: 225-239 (1989).
- Guengerich, F. P., and Shimada, T. Oxidation of toxic and human cytochrome P-450 enzymes. Chem. Res. Toxicol. 4: 391-407 (1991).
- Porter, T. D., and Coon, M. J. Cytochrome P-450. Multiplicity of isoforms, substrates, and catalytic and regulatory mechanisms. J. Biol. Chem. 266: 13469-13472 (1991).
- Hutzinger, O., Nash, D. M., Safe, S., Norstrom, R. J., Wildish, D. J., and Zitko, V. Polychlorinated biphenyls: metabolic behavior of pure isomers in pigeons, rats and brook trout. Science 178: 312-314 (1972).
- Sundstrom, G., Hutzinger, O., and Safe, S. The metabolism of chlorobiphenyls—a review. Chemosphere 5: 267-298 (1976).
- Schnellmann, R. G., Vickers, E. M., and Sipes, I. G. Metabolism and disposition of polychlorinated biphenyls. In: Reviews in Biochemical Toxicology, Vol. 7 (E. Hodgson, J. R. Bend, and R. M. Philpot, Eds.), Elsevier Press, Amsterdam, 1985, pp. 247-282.
- Sipes, I. G., and Schnellmann, R. G. Biotransformation of PCBs: metabolic pathways and mechanisms. In: Polychlorinated Biphenyls (PCBs). Mammalian and Environmental Toxicology. Environmental Toxin Series, Vol. 1 (S. Safe and O. Hutzinger, Eds.), Springer-Verlag, Heidelberg, 1987, pp. 97-110.
- Kaminsky, L. S., Kennedy, M. W., Adams, S. M., and Guengerich, F. P. Metabolism of dichlorobiphenyls by highly purified isozymes of rat liver cytochrome P-450. Biochemistry 20: 7379-7384 (1991).
- Preston, B. D., Miller, J. A., and Miller, E. C., Non-arene oxide aromatic ring hydroxylatic of 2,2',5,5'-tetrachlorobiphenyl as the major metabolic pathway catalyzed by phenobarbital-induced rat liver microsomes. J. Biol. Chem. 258: 8304-8311 (1983).
- Wyndham, C., and Safe, S. *In vitro* metabolism of 4-chlorobiphenyl by control and induced rat liver microsomes. Biochemistry 17: 208-215 (1978).
- Wong, A., Basur, P. K., and Safe, S. The metabolically mediated DNA damage and subsequent repair by 4-chlorobiphenyl in Chinese hamster ovary cells. Res. Commun. Chem. Pathol. Pharmacol. 24: 431-570 (1979).
- Shimada, T. Metabolic activation of [¹⁴C]polychlorinated biphenyl mixtures by rat liver microsomes. Bull. Environ. Contam. Toxicol. 16: 25-32 (1976).
- Shimada, T., and Sato, R. Covalent binding *in vitro* of polychlorinated biphenyls to microsomal macromolecules. Biochem. Pharmacol. 27: 585-593 (1978).
- Morales, N. M., and Matthews, H. B. *In vivo* binding of 2,3,6,2',3',6'-hexachlorobiphenyl and 2,4,5,2',4',5'-hexachlorobiphenyl to mouse liver macromolecules. Chem.-Biol. Interact. 27: 94-110 (1979).
- Stadnicki, S., Lin, F. S. D., and Allen, J. R. DNA single strand breaks caused by 2,2',5,5'-tetrachlorobiphenyl and its metabolites. Res. Commun. Chem. Pathol. Pharmacol. 24: 313-327 (1979).
- Schoeny, R. Mutagenicity testing of chlorinated biphenyls and chlorinated dibenzofurans. Mutat. Res. 101: 45-56 (1982).
- Bergman, A., Biessmann, A., Brandt, I., and Rafter, J. Metabolism of 2,4,5-trichlorobiphenyl: role of the intestinal microflora in the formation of bronchial-seeking methylsulphone metabolites in mice. Chem.-Biol. Interact. 40: 123-131 (1982).
- Baake, J. E., Bergman, A., and Larsen, G. L. Metabolism of 2,4',5-trichlorobiphenyl by the mercapturic acid pathway. Science 217: 645-647 (1982).
- Bergman, A., Brandt, I., and Jansson, B. Accumulation of methylsulfonyl derivatives of some bronchial-seeking polychlorinated biphenyls in the respiratory tract of mice. Toxicol. Appl. Pharmacol. 48: 213-220 (1979).
- Brandt, I. PCB methylsulphones and related compounds: identification of target cells and tissues in different species. Chemosphere 16: 1671-1676 (1987).
- Brandt, I., Klasson-Wehler, E., Rafter, J., and Bergman, A. Metabolism of 2,4',5-trichlorobiphenyl: tissue concentrations of methylsulphonyl-2,4',5-trichlorobiphenyl in germfree and conventional mice. Toxicol. Lett. 12: 273-280 (1982).
- Gillner, M., Lund, J., Cambillau, C., Alexandersson, M., Hurtig, U., Bergman, A., Klasson-Wehler, E., and Gustafsson, J.-A. The binding of methylsulfonyl-polychloro-biphenyls to uteroglobin. J. Steroid Biochem. 31: 27-33 (1988).
- Lund, J., Andersson, O., and Ripe, E. Characterization of a binding protein for the PCB metabolite 4,4'-bis(methylsulfonyl)-2,2',5,5'-tetrachlorobiphenyl present in bronchoalveolar lavage from healthy smokers and non-smokers. Toxicol. Appl. Pharmacol. 83: 486-493 (1986).
- Haraguchi, H., Kuroki, K., Masuda, Y., and Shigematsu, N. Determination of methylthio and methylsulphone polychlorinated biphenyls in tissues of patients with "Yusho". Food Chem. Toxicol. 22: 283-288 (1984).
- Warshaw, R., Fischbein, A., Thornton, J., Miller, A., and Selikoff, I. J. Decrease in vital capacity in PCB-exposed workers in a capacitor manufacturing facility. Ann. N. Y. Acad. Sci. 320: 277-283 (1979).
- Jansson, B., Jensen, S., Olsson, M., Renberg, L., Sundström, G., and Vaz, R. Identification by GC-MS of phenolic metabolites of PCB and p,p'-DDE isolated from Baltic guillemot and seal. Ambio 4: 93-97 (1975).
- Narasimhan, T. R., Kim, H. L., and Safe, S. Effects of hydroxylated polychlorinated biphenyls on mouse liver mitochondrial oxidative phosphorylation. J. Biochem. Toxicol. 6: 229-236 (1991).
- Yoshimura, H., Yonemoto, Y., Yamada, H., Koga, N., Oguri, K. and Saeki, S. Metabolism *in vivo* of 3,4,3',4'-tetrachlorobiphenyl and toxicological assessment of the metabolites in rats. Xenobiotica 17: 897-910 (1987).
- Shimada, T. Lack of correlation between formation of reactive metabolites

- and thymic atrophy caused by 3,4,3',4'-tetrachlorobiphenyl in C57BL/6N mice. *Arch. Toxicol.* 59: 301-306 (1987).
42. Koga, N., Beppu, M., and Yoshimura, H. Metabolism *in vivo* of 3,4,5,3',4'-pentachlorobiphenyl and toxicological assessment of the metabolite in rats. *Pharmacobio-Dyn* 13: 497-506 (1990).
 43. Safe, S. Halogenated hydrocarbons and aryl hydrocarbons identified in human tissues. *Toxicol. Environ. Chem.* 5: 153-165 (1982).
 44. Kimbrough, R. D. The toxicity of polychlorinated polycyclic compounds and related chemicals. *CRC Crit. Rev. Toxicol.* 2: 445-498 (1974).
 45. Kimbrough, R. D. Laboratory and human studies on polychlorinated biphenyls (PCBs) and related compounds. *Environ. Health Perspect.* 59: 99-106 (1985).
 46. Safe, S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *C.R.C. Crit. Rev. Toxicol.* 21: 51-88 (1990).
 47. Silberhorn, E. M., Glauert, M. P., and Robertson, L. W. Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. *CRC Crit. Rev. Toxicol.* 20: 439-496 (1990).
 48. Norback, D. H., and Weltman, R. H. Polychlorinated biphenyl induction of hepatocellular carcinoma in the Sprague-Dawley rat. *Environ. Health Perspect.* 60: 97-105 (1985).
 49. Schaeffer, E., Greim, H., and Goessner, W. Pathology of chronic polychlorinated biphenyl (PCB) feeding in rats. *Toxicol. Appl. Pharmacol.* 75: 278-288 (1984).
 50. Allen, J. R. Response of the nonhuman primate to polychlorinated biphenyl exposure. *Fed. Proc.* 34: 1675-1679 (1975).
 51. McNulty, W. P. Toxicity and fetotoxicity of TCDD, TCDF, and PCB isomers in rhesus macaques (*Macaca mulatta*). *Environ. Health Perspect.* 60: 77-88 (1985).
 52. Platonow, N. S., and Karstad, L. K. Dietary effect of polychlorinated biphenyls on mink. *Can. J. Comp. Med.* 37: 391-400 (1973).
 53. Ringer, R. K., Aulerich, R. J., and Bleavins, M. R. Biological effects of PCBs and PBBs on mink and ferrets—a review. In: *Halogenated Hydrocarbons: Health and Ecological Effects* (M. A. Q. Khan, Ed.), Pergamon Press, New York, 1991. pp. 329-343.
 54. Parkinson, A., Safe, S., Robertson, L., Thomas, P. E., Ryan, D. E., and Levin, W. Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated and polybrominated biphenyls: a study of structure activity relationships. *J. Biol. Chem.* 258: 5967-5976 (1983).
 55. Lubet, R. A., Jones, C. R., Stockus, D. L., Fox, S. D., and Nims, R. W. Induction of cytochrome P450 and other drug metabolizing enzymes in rat liver following dietary exposure to Aroclor 1254. *Toxicol. Appl. Pharmacol.* 108: 355-365 (1991).
 56. Makary, M., Kim, H. L., Safe, S., Womack, J., and Ivie, G. W. Constitutive and Aroclor 1254-induced hepatic glutathione S-transferase, peroxidase and reductase activities in genetically inbred mice. *Comp. Biochem. Physiol.* 91: 425-429 (1988).
 57. Safe, S., Bandiera, S., Sawyer, T., Robertson, L., Parkinson, A., Thomas, P. E., Ryan, D. E., Reik, L. M., Levin, W., Denomme, M. A., and Fujita, T. PCBs: structure-function relationships and mechanism of action. *Environ. Health Perspect.* 60: 47-56 (1985).
 58. Safe, S., Bandiera, S., Sawyer, T., Zmudzka, B., Mason, G., Romkes, M., Denomme, M. A., Sparling, J., Okey, A. B., and Fujita, T. Effects of structure on binding to the 2,3,7,8-TCDD receptor protein and AHH induction—halogenated biphenyls. *Environ. Health Perspect.* 61: 21-33 (1985).
 59. Conney, A. H. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19: 317-366 (1967).
 60. Conney, A. H. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res.* 42: 4875-4917 (1982).
 61. Snyder, R., and Remmer, H. Classes of hepatic microsomal mixed function oxidase inducer. *Pharmacol. Ther.* 7: 203-244 (1979).
 62. Alvares, A. P., Bickers, D. R., and Kappas, A. Polychlorinated biphenyls: a new type of inducer of cytochrome P-448 in the liver. *Proc. Natl. Acad. Sci. U.S.A.* 70: 1321-1325 (1973).
 63. Alvares, A. P., and Kappas, A. Heterogeneity of cytochrome P-450s induced by polychlorinated biphenyls. *J. Biol. Chem.* 252: 6373-6378 (1977).
 64. Alvares, A. P., and Kappas, A. The inducing properties of polychlorinated biphenyls on hepatic monooxygenases. *Clin. Pharmacol. Ther.* 22: 809-816 (1977).
 65. Ryan, D. E., Thomas, P. E., and Levin, W. Properties of purified liver microsomal cytochrome P-450 from rats treated with the polychlorinated biphenyl mixture Aroclor 1254. *Mol. Pharmacol.* 13: 521-532 (1977).
 66. Ryan, D. E., Thomas, P. E., Korzeniowski, D., and Levin, W. Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. *J. Biol. Chem.* 254: 1365-1374 (1979).
 67. Botelho, L. H., Ryan, D. E., and Levin, W. Amino acid compositions and partial amino acid sequences of three highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, or 3-methylcholanthrene. *J. Biol. Chem.* 254: 5635-5640 (1979).
 68. Thomas, P. E., Reik, L. M., Ryan, D. E., and Levin, W. Regulation of three forms of cytochrome P-450 and epoxide hydrolase in rat liver microsomes: effects of age, sex and induction. *J. Biol. Chem.* 256: 1044-1052 (1981).
 69. Yoshimura, H., Yoshihara, S., Ozawa, N., and Miki, M. Possible correlation between induction modes of hepatic enzymes by PCBs and their toxicity in rats. *Ann. N. Y. Acad. Sci.* 320: 179-182 (1970).
 70. Poland, A., and Glover, E. Chlorinated biphenyl induction of aryl hydrocarbon hydroxylase activity: a study of the structure activity relationship. *Mol. Pharmacol.* 13: 924-938 (1977).
 71. Goldstein, J. A., Hickman, P., Bergman, H., McKinney, J. D., and Walker, M. P. Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. *Chem.-Biol. Interact.* 17: 69-87 (1977).
 72. Kohli, K. K., Philnot, R. M., Albro, P. W., and McKinney, J. D. Induction of different species of cytochrome P-450 by coplanar and non-coplanar isomers of hexachlorobiphenyl. *Life Sci.* 26: 945-952 (1980).
 73. Parkinson, A., Robertson, L., Safe, L., and Safe, S. Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: structure-activity rules. *Chem.-Biol. Interact.* 30: 271-285 (1981).
 74. Poland, A., Greenlee, W. E., and Kende, A. S. Studies on the mechanism of action of the chlorinated dibenzo-*p*-dioxins and related compounds. *Ann. N. Y. Acad. Sci.* 320: 214-230 (1979).
 75. Safe, S., Robertson, L. W., Safe, L., Parkinson, A., Bandiera, S., Sawyer, T., and Campbell, M. A. Halogenated biphenyls: molecular toxicology. *Can. J. Physiol. Pharmacol.* 60: 1057-1064 (1982).
 76. Parkinson, A., and Safe, S. Mammalian biologic and toxic effects of PCBs. In: *Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology*, Environmental Toxin Series, Vol. 1 (S. Safe and O. Hutzinger, Eds.), Springer-Verlag, Heidelberg, 1987. pp. 49-75.
 77. Bandiera, S., Safe, S., and Okey, A. B. Binding of polychlorinated biphenyls classified as either PB-, MC- or mixed-type inducers to cytosolic Ah receptor. *Chem.-Biol. Interact.* 39: 259-278 (1983).
 78. Dannan, G. A., Moore, R. W., Besaw, L. C., Aust, S. D. 2,4,5,3',4',5'-Hexabromobiphenyl is both a 3-methylcholanthrene- and a phenobarbital-type inducer of microsomal drug metabolizing enzymes. *Biochem. Biophys. Res. Commun.* 85: 450-458 (1978).
 79. Parkinson, A., Cockerline, R., and Safe, S. Induction of both 2-methylcholanthrene and phenobarbitone-type microsomal enzyme activity by a single polychlorinated biphenyl isomer. *Biochem. Pharmacol.* 29: 259-262 (1980).
 80. Parkinson, A., Cockerline, R., and Safe, S. Polychlorinated biphenyl isomers and congeners as inducers of both 3-methylcholanthrene- and phenobarbitone-type microsomal enzyme activity. *Chem.-Biol. Interact.* 29: 277-289 (1980).
 81. Yamamoto, H., Yoshimura, H., Fujita, M., and Yamamoto, T. Metabolic and toxicologic evaluation of 2,3,4,4',5'-pentachlorobiphenyl in rats and mice. *Chem. Pharm. Bull.* 24: 2168-2174 (1976).
 82. Leece, B., Denomme, M. A., Towner, R., and Li, S. M. A. Polychlorinated biphenyls: correlation between *in vivo* and *in vitro* quantitative structure-activity relationships (QSARs). *J. Toxicol. Environ. Health* 16: 379-388 (1985).
 83. Ax, R. L., and Hansen, L. G. Effects of purified PCB analogs on chicken reproduction. *Poultry Sci.* 54: 895-900 (1975).
 84. Yoshihara, S., Kawano, K., Yoshimura, H., Kuroki, H., and Masuda, Y. Toxicological assessment of highly chlorinated biphenyl congeners retained in the Yusho patients. *Chemosphere* 8: 531-538 (1979).
 85. Davis, D., and Safe, S. Immunosuppressive activities of polychlorinated biphenyls in C57BL/6 mice: structure-activity relationships as Ah receptor agonists and partial antagonists. *Toxicology* 63: 97-111 (1990).
 86. Silkworth, J. B., and Grabstein, E. M. Polychlorinated biphenyl immunotoxicity: dependence on isomer planarity and Ah gene complex. *Toxicol. Appl. Pharmacol.* 65: 109-115 (1982).

87. Parkinson, A., Robertson, L., Uhlig, L., Campbell, M. A., and Safe, S. 2,3,4,4',5-Pentachlorobiphenyl: differential effects on C57BL/6J and DBA/2J inbred mice. *Biochem. Pharmacol.* 31: 2830-2833 (1982).
88. Robertson, L., Parkinson, A., Bandiera, S., Campbell, M. A., Lambert, I., Merrill, J., and Safe, S. PCBs and PBBs—biologic and toxic effects on C57BL/6J and DBA/2J inbred mice. *Toxicology* 31: 191-206 (1984).
89. Stonard, M. D., and Grieg, J. B. Different patterns of hepatic microsomal enzyme activity produced by administration of pure hexachlorobiphenyl isomers and hexachlorobenzene. *Chem.-Biol. Interact.* 15: 365-179 (1976).
90. Parkinson, A., Robertson, L., Safe, L., and Safe, S. Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: effects of diortho substitution. *Chem.-Biol. Interact.* 31: 1-12 (1981).
91. Parkinson, A., Robertson, L., and Safe, S. Hepatic microsomal enzyme induction by 2,2',3,3',4,4'- and 2,2',3,4,4',5-hexachlorobiphenyl. *Life Sci.* 27: 2333-2337 (1980).
92. U.S. EPA. Workshop Report on Toxicity Equivalency Factors for Polychlorinated Biphenyl Congeners. Risk Assessment Forum, EPA/625/3-91/020, U.S. Environmental Protection Agency, Washington, DC, 1991.
93. Tanabe, S. A need for reevaluation of PCB toxicity. *Mar. Pollut. Bull.* 20: 247-248 (1989).
94. Denomme, M. A., Bandiera, S., Lambert, I., Copp, L., Safe, L., and Safe, S. Polychlorinated biphenyls as phenobarbitone-type inducers of microsomal enzymes—structure activity relationships for a series of 2,4-dichloro-substituted congeners. *Biochem. Pharmacol.* 32:2955-2963 (1983).
95. Buchmann, A., Kunz, W., Wolf, C. R., Oesch, F., and Robertson, L. W. Polychlorinated biphenyls, classified as either phenobarbital- or 3-methylcholanthrene-type inducers of cytochrome P-450, are both hepatic tumor promoters in diethylnitrosamine-initiated rats. *Cancer Lett.* 32: 243-253 (1986).
96. Robertson, L. W., Silberhorn, E. M., and Glauert, H. P. Do structure-activity relationships for the acute toxicity of PCBs and PBBs also apply for induction of hepatocellular carcinoma? *Environ. Toxicol. Chem.* 10: 715-726 (1991).
97. Scheutz, E. G., Wrigton, S. A., Safe, S., and Guzelian, P. S. Regulation of cytochrome P-450p by phenobarbital and phenobarbital-like inducers in adult rat hepatocytes in primary monolayer culture. *In Vivo Biochem.* 25: 1124-1133 (1986).
98. Lilienthal, H., Neuf, M., Munoz, C., and Winneke G. Behavioral effects of pre- and postnatal exposure to a mixture of low chlorinated PCBs in rats. *Fundam. Appl. Toxicol.* 15: 457-467 (1990).
99. Seegal, R. F., Brosch, K. O., and Bush, B. Polychlorinated biphenyls produce regional alterations of dopamine metabolism in rat brain. *Toxicol. Lett.* 30: 197-202 (1986).
100. Schantz, S. L., Levin, E. D., and Bowman, R. E. Long-term neuro-behavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys. *Environ. Toxicol. Chem.* 10: 747-756 (1991).
101. Lilienthal, H., and Winneke, G. Sensitive periods for behavioral toxicity of polychlorinated biphenyls: determination by cross-fostering in rats. *Fundam. Appl. Toxicol.* 17: 368-375 (1991).
102. Tilson, H. A., Davis, G. J., McLachlan, J. A., and Lucier, G. W. The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. *Environ. Res.* 18: 466-474 (1979).
103. Eriksson, P. Effects of 3,3',4,4'-tetrachlorobiphenyl in the brain of the neonatal mouse. *Toxicology* 49: 43-48 (1988).
104. Eriksson, P., Lundkvist, U., and Fredriksson, A. Neonatal exposure to 3,3',4,4'-tetrachlorobiphenyl: changes in spontaneous behaviour and cholinergic muscarinic receptors in the adult mouse. *Toxicology* 69: 27-14 (1991).
105. Seegal, R. F., Bush, B., and Shain, W. Lightly chlorinated ortho-substituted PCB congener decrease dopamine in nonhuman primate brain and in tissue culture. *Toxicol. Appl. Pharmacol.* 106: 136-144 (1990).
106. Shain, W., Bush, B., and Seegal, R. Neurotoxicity of polychlorinated biphenyls: structure-activity relationship of individual congeners. *Toxicol. Appl. Pharmacol.* 111: 33-42 (1991).
107. Safe, S. PCBs and human health. In: *Polychlorinated Biphenyls (PCBs): Mammalian Environmental Toxicology, Environmental Toxin Series, Vol. 1* (S. Safe and O. Hutzinger, Eds.), Springer-Verlag, Heidelberg, 1987, pp. 1-13.
108. Kimbrough, R. D. Human health effects of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). *Annu. Rev. Pharmacol. Toxicol.* 27: 87-111 (1987).
109. Takamatsu, M., Oki, M., Maeda, K., Inoue, Y., Hirayama, H., and Yoshizuka, K. Surveys of workers occupationally exposed to PCBs and of Yusho patients. *Environ. Health Perspect.* 59: 91-97 (1985).
110. Kreiss, K. Studies on populations exposed to polychlorinated biphenyls. *Environ. Health Perspect.* 60: 193-199 (1985).
111. Chase, K. H., Wong, O., Thomas, D., Stal, B. W., and Simon, R. K. Clinical and metabolic abnormalities associated with occupational exposure to polychlorinated biphenyls. *J. Occup. Med.* 24: 109-114 (1982).
112. Lawton, R. W., Ross, M. R., Feingold, J., and Brown, J. F. Effects of PCB exposure on biochemical and hematological findings in capacitor workers. *Environ Health Perspect.* 60: 165-184 (1985).
113. Emmett, E. A. Polychlorinated biphenyl exposure and effect in transformer repair workers. *Environ Health Perspect.* 60: 185-192 (1985).
114. Meigs, J. W., Albom, J. J., and Kartin, B. I. Chloracne from an unusual exposure to Aroclor. *J. Am. Med. Assoc.* 154: 1417-1418 (1954).
115. Ouw, H. K., Simpson, G. R., and Siyali, D. S. Use and health effects of Aroclor 1242, a polychlorinated biphenyl in an electrical industry. *Arch. Environ. Health* 31: 189-194 (1976).
116. Alvares, A. P., Fischbein, A., Anderson, K. E., and Kappas, A. Alterations in drug metabolism in workers exposed to polychlorinated biphenyls. *Clin. Pharmacol. Ther.* 22: 140-146 (1977).
117. Roach, J. A. G., and Pomerantz, I. H. The finding of chlorinated dibenzofurans in a Japanese polychlorinated biphenyl sample. *Bull. Environ. Contam. Toxicol.* 12: 338-342 (1974).
118. Bowes, G. W., Mulvihill, M. J., Simoneit, B. R. T., Burlingame, A. L., and Risebrough, R. W. Identification of chlorinated dibenzofurans in American polychlorinated biphenyls. *Nature* 256: 305-307 (1975).
119. Buser, H. R., Rappe, C., and Gara, A. Polychlorinated dibenzofurans (PCDFs) found in Yusho oil and in used Japanese PCB. *Chemosphere* 5: 439-449 (1978).
120. Miyata, H., and Kashimoto, T. Studies on the compounds related to PCB (TV). Investigation on polychlorodibenzofuran formation. *J. Food Hyg. Soc.* 19: 78-84 (1978).
121. Brown, D. P., and Jones, M. Mortality and industrial hygiene study of workers exposed to polychlorinated biphenyls. *Arch. Environ. Health* 36: 120-129 (1981).
122. Brown, D. P. Mortality of workers exposed to polychlorinated biphenyls - an update. *Arch. Environ. Health* 42: 333-339 (1987).
123. Bertazzi, P. A., Riboldi, L., Pesatori, A., Radice, L., and Zocchetti, C. Cancer mortality of capacitor manufacturing workers. *Am. J. Ind. Med.* 11: 165-176 (1987).
124. Gustavsson, P., Hogstedt, C., and Rappe, C. Short-term mortality and cancer incidence in capacitor manufacturing workers exposed to polychlorinated biphenyls (PCBs). *Am. J. Ind. Med.* 10: 341-344 (1986).
125. Taylor, P. R., Lawrence, C. E., Hwang, J. -L., and Paulson, A. S. Polychlorinated biphenyls: influence on birthweight and gestation. *Am. J. Public Health* 74: 1153-1154 (1984).
126. Taylor, P. R., Stelma, J. M., and Lawrence, C. E. The relation of polychlorinated biphenyls to birth weight and gestational age in the offspring of occupationally exposed mothers. *Am. J. Epidemiol.* 129: 395-406 (1989).
127. Fein, G. G., Jacobson, J. L., Jacobson, S. W., Schwartz, P. M., and Dowler, J. K. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. *J. Pediatr.* 105: 315-320 (1984).
128. Rogan, W. J., Gladen, B. C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J., Tingelstad, J., and Tully, M. Neonatal effects of transplacental exposure to PCBs and DDE. *J. Pediatr.* 335-341 (1986).
129. Jacobson, J. L., Jacobson, S. W., Fein, G. G., Schwartz, P. M., Dowler, J. K. Prenatal exposure to an environmental toxin: a test of the multiple effects model. *Dev. Psychol.* 20: 523-532 (1984).
130. Gladen, B. C., Rogan, W. J., Hardy, P., Thullen, J., Tingelstad, J., and Tully, M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. *J. Pediatr.* 113: 991-995 (1988).
131. Jacobson, S. W., Fein, G. G., Jacobson, J. L., Schwartz, P. M., and Dowler, J. K. The effect of PCB exposure on visual recognition memory. *Child Dev.* 56: 853-860 (1985).
132. Jacobson, J. L., Jacobson, S. W., and Humphrey, H. E. B. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. *J. Pediatr.* 116: 38-45 (1990).
133. Jacobson, J. L., Jacobson, S. W., and Humphrey, H. E. B. Effects of exposure to PCBs and related compounds on growth and activity in children. *Neurotoxicol. Teratol.* 12: 319-326 (1990).

134. Rogan, W. J., Gladen, B. C., Hung, K., Koong, S., Shih, L., J. Taylor, J. S., Wu, Y., Yang, D., Ragan, N. B. and Hsu, C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science* 241: 334-366 (1988).
135. Bellin, J. S., and Barnes, Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxins and -Dibenzofurans (CDDs and CDFs), 2nd ed. U.S. Environmental Protection Agency, Washington, DC 1989.
136. Ahlborg, U. G., Hakansson, H., Waern, F., and Hanberg, A. Nordisk dioxinriskbed-mning. Rapport fran en nordisk expertgrupp. Nordisk Ministerratet, Miljorapport, Denmark, 1988.
137. NATO/CCMS. Pilot Study on International Information Exchange on Dioxins and Related Compounds. North Atlantic Treaty (NATO)/Committee on the Challenges of Modern Society (CCMS), Report Number 176, Washington, DC, 1988.
138. Kutz, F. W., Barnes, D. G., Bretthauer, E. W., Bottimore, D. P., and Greim, H. The international toxicity equivalency factor (I-TEF) method for estimating risks associated with exposures to complex mixtures of dioxins and related compounds. *Toxicol. Environ. Chem.* 26: 99-109 (1990).
139. Ahlborg, U. G. Nordic risk assessment of PCDDs and PCDFs. *Chemosphere* 19: 603-608 (1989).
140. Tanabe, S., Kannan, N., Wakimoto, T., Tatsukawa, R., Okamoto, T., and Masuda, Y. Isomer-specific determination and toxic evaluation of potentially hazardous coplanar PCBs, dibenzofurans and dioxins in the tissues of "Yusho" PCB poisoning victim and in the causal oil. *Toxicol. Environ. Chem.* 24: 215-231 (1989).
141. Kannan, N., Tanabe, S., Ono, M., and Tatsukawa, R. Critical evaluation of polychlorinated biphenyl toxicity in terrestrial and marine mammals: increasing impact of non-*ortho* and mono-*ortho* coplanar polychlorinated biphenyls from land to ocean. *Arch. Environ. Contam. Toxicol.* 18: 850-857. (1989).
142. Kannan, N., Tanabe, S., and Tatsukawa, R. Toxic potential of non-*ortho* and mono-*ortho* coplanar PCBs in commercial PCBs in commercial PCB preparations: 2,3,7,8-TCDD toxicity equivalence factors approach. *Bull. Environ. Contam. Toxicol.* 41: 267-276 (1988).
143. Kannan, N., Tanabe, S., and Tatsukawa, R. Potentially hazardous residues of non-*ortho* chlorine substituted coplanar PCBs in human adipose tissue. *Arch. Environ. Health* 43: 11-14 (1988).
144. Kannan, N., Tanabe, S., Wakimoto, T., and Tatsukawa, R. Coplanar polychlorinated biphenyls in Aroclor and Kanechlor mixtures. *J. Assoc. Off. Anal. Chem.* 70: 451 (1987).
145. Sawyer, T. and Safe, S. PCB isomers and congeners: induction of aryl hydrocarbon hydroxylase and ethoxyresorufin *O*-deethylase enzyme activities in rat hepatoma cells. *Toxicol. Lett.* 13: 87-94 (1982).
146. Dewailly, E., Weber, J.-P., Gingras, S., and Laliberte, C. Coplanar PCBs in human milk in the province of Quebec, Canada: are they more toxic than dioxin for breast fed infants? *Bull. Environ. Contam. Toxicol.* 47: 491-498 (1991).
147. Kubiak, T. J., Harris, H. J., Smith, L. M., Schwartz, T. R., Stalling, Trick, J. A., Sileo, L., Docherty, D. E., and Erdman, T. C. Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan-1983. *Arch. Environ. Contam. Toxicol.* 18: 706-727 (1989).
148. Tillitt, D. E., Ankley, G. T., Verbrugge, D. A., Giesy, J. P., Ludwig, J. P., and Kubiak, T. J. H4IIE rat hepatoma cell bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in colonial fish-eating waterbird eggs from the Great Lakes. *Arch. Environ. Contam. Toxicol.* 21: 91-101 (1990).
149. Tillitt, D. E. and Giesy, J. P. Characterization of the H4IIE rat hepatoma cell bioassay as a tool for assessing toxic potency of planar halogenated hydrocarbons in environmental samples. *Environ. Sci. Technol.* 25: 87-92 (1991).
150. Smith, L. M., Schwartz, T. R., and Feltz, K. Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachloro-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota. The question of their relative toxicological significance. *Chemosphere* 9: 1063-1085 (1990).
151. Giesy, J. P., Jones, P. D., Mora, M., Newsted, J. L., Verbrugge, D. A., Aulerich, R., Bursian, S., Summers, C., Ludwig, J. P., Ludwig, M. J., Kubiak, T. J., Best, D. A., Ankley, G. T., Lodge, K. B., Tillitt, D. E., Stromberg, K., and Tanabe, S. The use of TLFs for understanding the toxicology of complex mixtures of chlorinated hydrocarbons in fish and wildlife of the Great Lakes (abstract). In: *Dioxin '91 Abstracts*, 1991, p. 24.
152. Mac, M. J., and Schwartz, Investigations into the effects of PCB congeners on reproduction in lake trout from the Great Lakes (abstract). In: *Dioxin '91 Abstracts*, 1991, p. 64.
153. Giesy, J. P., Jones, P. D., Mora, M., Newsted, J. L., Verbrugge, D. A., Aulerich, R., Bursian, S., Ludwig, J. P., Ludwig, M. J., Kubiak, T. J., Best, D. A., Ankley, G. T., Lodge, K. B., Tillitt, D. E., Stromberg, K., and Tanabe, S. Relative concentrations of coplanar PCB, chlorinated dibenzodioxin (PCDD) and dibenzofuran (PCDF) congeners in fish and wildlife of the North American Great Lakes and their relationships with observed effects (abstract). In: *Dioxin '91 Abstracts*, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, 1991, p. 63.